

CLAIMS

1. A solid support having immobilised thereon a plurality of oligonucleotides at pre-defined positionally distinct sites, characterised in that the sequence of each oligonucleotide that binds to its complementary sequence has substantially the same melting temperature (T_m).
2. A support as claimed in claim 1 wherein 90% of the oligonucleotides on the support possess T_m s within 4°C of each other.
3. A support as claimed in claim 1 or 2 wherein the oligonucleotides are detectably labelled.
4. A support as claimed in any of the preceding claims which comprises at least 50, particularly at least 500 and more particularly at least 5000, distinct oligonucleotides on the support.
5. A support as claimed in any of claims 1 to 4 wherein each oligonucleotide is non-complementary with genomic DNA and non complementary with each other.
6. A method for identifying the presence or absence of one or more test nucleic acid sequences in a sample, comprising:
 - (i) contacting a nucleic acid containing sample with a plurality of single stranded targeting polynucleotide molecules under suitable hybridisation conditions to ensure hybrid formation between the targeting nucleotide portion of the targeting polynucleotide molecule and its complementary target nucleic acid sequence in the sample, each of said targeting polynucleotide molecules possessing, in addition to the targeting nucleotide portion, a unique single-stranded oligonucleotide tail sequence complementary to a unique capture oligonucleotide sequence attached to a solid support, characterised in that substantially all of the oligonucleotides possess substantially the same T_m when bound to their complementary sequence on the tail;
 - (ii) optionally separating the unhybridised targeting polynucleotide molecules from the hybrid molecules;
 - (iii) contacting the population of hybrid molecules to a solid support having attached thereon at pre-defined locations unique capture oligonucleotides, each capture oligonucleotide being complementary to one or other of the oligonucleotide tail

sequences on the targeting molecules, under suitable conditions to ensure capture of each of the hybrid molecules to the solid support; and

(iv) determining the presence or absence of the captured hybrid molecules at each of the pre-defined locations on the solid support.

- 5 7. A method as claimed in claim 6 wherein the targeting polynucleotides are amplification primers and an amplification reaction is performed after step (i).
8. A method as claimed in claim 7 wherein the primers are amplification refractory mutation system (ARMS) primers.
9. A method as claimed in claim 7 or claim 8 wherein each primer is used in conjunction
10 with a second companion primer to amplify the target region of interest.
10. A method for identifying the differential expression of each of a plurality of genes in a first cell type with respect to expression of the same genes in a second cell type comprising:
 - (i) isolating mRNA from each cell type and converting said mRNA into cDNA with
15 incorporation of a detectable label into the newly synthesised cDNA;
 - (ii) optionally, fragmenting said newly synthesised cDNA into appropriate length nucleic acid fragments;
 - (iii) contacting said labelled nucleic acid with a plurality of targeting polynucleotide molecules under suitable conditions to effect hybridisation between substantially
20 complementary sequences, each polynucleotide molecule comprising a unique 3' portion substantially complementary to a unique target nucleic acid sequence which may be present in a sample, a 5' tail portion complementary to one of a group of pre-selected oligonucleotides that each possess substantially the same melting temperature (T_m) and are attached at pre-defined positions to a solid support, and optionally a spacer moiety
25 interposed between said 3' and 5' portions;
 - (iv) contacting the hybridised products from step (iii) to a solid surface on which is immobilised at pre-determined positions a plurality of oligonucleotide sequences complementary to one or other of the 5' tail portions of the targeting polynucleotides; and,
 - 30 (v) determining the expression level of each gene in each cell type according to the amount of label detected.

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11. A method as claimed in claim 10 wherein each target gene is detected by a plurality of targeting polynucleotides that bind at distinct parts of the target gene.
12. A method as claimed in claim 10 or claim 11 wherein there are between 5 and 80 distinct targeting oligonucleotides per target gene.
- 5 13. A method as claimed in claim 10, 11 or 12 wherein step (iii) is carried out as separate reactions between the target cDNAs and separate pools of targeting polynucleotides, the cDNA target binding sequences of the polynucleotides in each pool possessing approximately the same T_m as the others in the pool; the hybridisation reactions from each pool of targeting polynucleotides are then pooled together.
- 10 14. A method for quantifying the expression level of a gene comprising:
 - (i) converting mRNA from a test sample into cDNA;
 - (ii) optionally, fragmenting said newly synthesised cDNA into appropriate length nucleic acid fragments;
 - (iii) contacting the cDNA with a plurality of targeting polynucleotide molecules under
 - 15 suitable conditions to allow hybridisation between substantially complementary sequences to occur, each polynucleotide molecule comprising a unique 3' portion substantially complementary to a unique region of the cDNA, a 5' tail portion complementary to one of a group of pre-selected oligonucleotides that are attached at pre-defined positions to a solid support, and optionally a spacer moiety interposed
 - 20 between said 3' and 5' portions;
 - (iv) contacting the components from step (iii) with a substrate on which is immobilised at pre-determined positions a plurality of capture oligonucleotide sequences each complementary to one or other of the 5' tail portions of the targeting polynucleotides so as to allow the tailed cDNA/targeting polynucleotide duplex molecules to bind to
 - 25 their complementary capture oligonucleotide on the substrate; and
 - (v) detecting the amount of bound cDNA/targeting polynucleotide duplex at each position on the substrate.
15. A kit for detecting the presence or absence of one or more target nucleic acid sequences contained in a sample, which kit comprises:-
 - 30 (i) a plurality of polynucleotides, each polynucleotide comprising a unique 3' portion substantially complementary to a unique target nucleic acid sequence which may be

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present in a sample, a 5' portion complementary to one of a group of pre-selected oligonucleotides that each possess substantially the same melting temperature (T_m) and are attached at pre-defined positions to a solid support, and optionally a spacer moiety interposed between said 3' portion and said 5' portion; and

- 5 (ii) a solid support having immobilised thereon a plurality of pre-selected oligonucleotides at pre-defined positionally distinct sites, characterised in that the composition of each of the oligonucleotides is such that they all have substantially the same melting temperature (T_m) when annealed to their complementary sequence, each capture oligonucleotide having a sequence complementary to a 5' portion of one of the
- 10 polynucleotides in (i).

16. A kit as claimed in claim 15 also comprising one or more of:

- (i) nucleotide triphosphates;
- (ii) a polymerisation agent;
- (iii) control DNA; and,
- 15 (iv) instructions for use.